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# What do we need to know about RAVs clinically ?

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MEDIZINISCHE KLINIK 1

# Background

- Resistance associated variants (RAVs) or resistance associated polymorphisms (RAPs) with reduced drug susceptibility are observed in patients with chronic HCV
  - Due to the high replication rate of HCV ($10^{12}$ / day)
  - Error-prone RNA-dependent RNA polymerase ($10^{-4}$ to $10^{-5}$ per copied nucleotide)
- All possible single and double mutants are predicted to be generated multiple times each day
- Not all RAVs are fit enough to persist (but potentially under selection pressure of a respective DAA). Most NS3A RAVs disappear after EoT (HCV1b > HCV1a), while many NS5A RAVs persist
- The level of resistance *in vivo* may differ between a RAV in previously untreated patient and the same RAV selected by a DAA (= TEV) (e.g. due to compensatory mutations)

Rong et al., Sci Transl Med 2010

# How to define a RAV ?

- RAV per specific drug or drug class
  (e.g. resistance against elbasvir or any NS5A inhibitor)
- Genotypic backbone *in vitro* and *in vivo*
  (e.g. genotype 1a or 1b replicons, other genotypes)

| Group | GT | Position | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | K24 | M28 (L28)† | Q30 (R30)* | L31 | P32 | S38 | H58 (P58)‡ | A92 | Y93 |
| EBR RAVs | 1a | -¶ | A, G, T | D, E, H, G K, L, R | F, M, V | -¶ | -¶ | D | -¶ | C, H, N, S |
| | 1b | -¶ | Any | Any | Any | L | -¶ | D | K | Any |
| NS5A Class RAVs | 1a or 1b | Any | Any | Any | Any | Any | Any | Any | Any | Any |

†Wild type amino acid at position 28 is M in GT1a and L in GT1b
*Wild-type amino acid at position 30 is Q in GT1a and R in GT1b.
‡Wild type amino acid at position 58 is H in GT1a and P in GT1b
¶No EBR RAVs identified at this position

**Note that EBR RAVs are a subset of the NS5A class RAVs**

Zeuzem et al., Ann Intern Med 2015
Jacobson et al., AASLD 2016

# How to define a RAV ?

- RAV per specific drug or drug class
  (e.g. resistance against ledipasvir or any NS5A inhibitor)
- Genotypic backbone *in vitro* and *in vivo*
  (e.g. genotype 1a or 1b replicons, other genotypes)
- Level of reduced susceptibility *in vitro*
  (e.g. any aa change vs >2.5-fold vs >100-fold)

# NS5A RAVs - Overview

| | GT 1a | | | | GT1b | |
|---|---|---|---|---|---|---|
| | M28T | Q30R | L31M/V | Y93H/N | L31V | Y93H/N |
| Ledipasvir | >20x | >100x | >100x | >1,000x | | >1,000x/- |
| Ombitasvir | >1,000x | >100x | <3x<br>>100x | >10,000x | <10x | >50x |
| Daclatasvir | >100x | >1,000x | >100x<br>>1000x | >1,000x<br>>10,000x | >20x | >20x |
| Elbasvir | >10x | >10x | >10x<br>>100x | >100x | <10x | >10x/- |
| Velpatasvir | <10x | <3x | >20x | >100x<br>>1,000x | | <3x/- |
| Odalasvir | >20x | <10x | <3x | >1,000x | <3x | <3x<br><10x |
| ABT-530 | <3x | <3x | <3x | <10x | <3x | <3x |
| MK-8408 | <10x | <10x | <10x | <10x | <10x | <10x |

$EC_{50}$ fold-change compared to WT replicon

Cheng et al., EASL 2012; Wong et al., AAC 2013; Krishnan et al., AAC 2015; Fridell et al., Hepatology 2011; Liu et al., AAC 2015; Cheng et al., EASL 2013; Patel et al., EASL 2015; Ng et al., CROI 2014; Asante-Appiah et al., AASLD 2014

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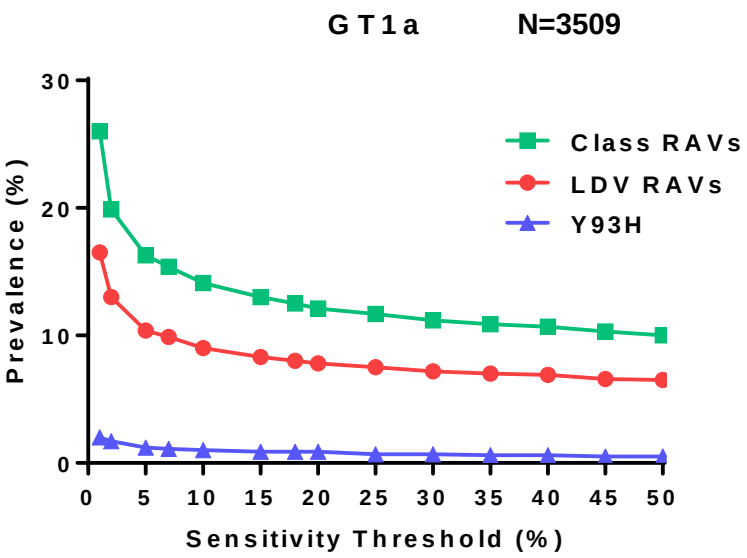
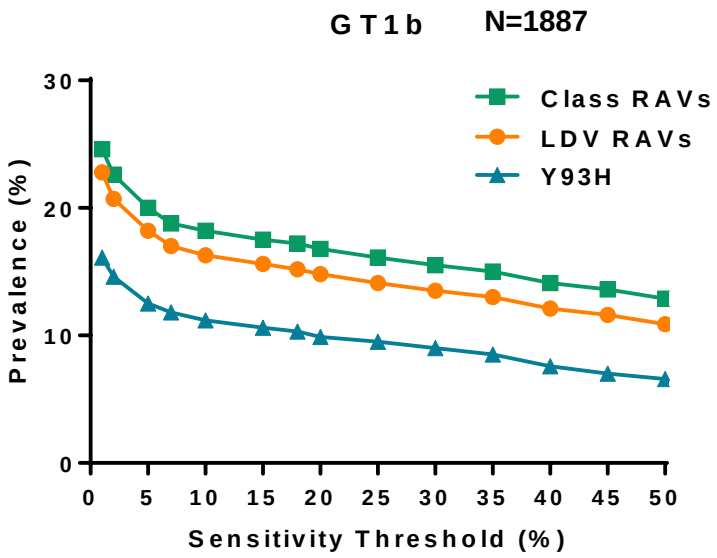
- Level of reduced susceptibility *in vitro*
  (e.g. any aa change vs >2.5-fold vs >100-fold)
- Methodology of RAV detection (sensitivity threshold)
  (e.g. deep sequencing with a cut-off at 1% or
  populations sequencing with a cut-off at 15-25%)

# Methodology of RAV detection




| GT1a Position | LDV RAVs | NS5A Class RAVs |
|---|---|---|
| 24 | G/N/R | G/N/R |
| 26 | | E |
| 28 | A/G/T | A/G/T/V |
| 30 | E/G/H/L/K/R/T | C/E/G/H/I/L/K/R/S/T/Y |
| 31 | I/F/M/V | I/F/M/V |
| 32 | L | L |
| 38 | F | F |
| 58 | D | D/L |
| 92 | K/T | K/T |
| 93 | C/F/H/N/S | C/F/H/L/N/R/S/T/W |

| GT1b Position | LDV RAVs | NS5A Class RAVs |
|---|---|---|
| 28 | | M |
| 31 | I/F/M/V | I/F/M/V |
| 32 | L | L |
| 58 | D | D |
| 92 | K | K |
| 93 | C/H/N/S | C/H/N/S |

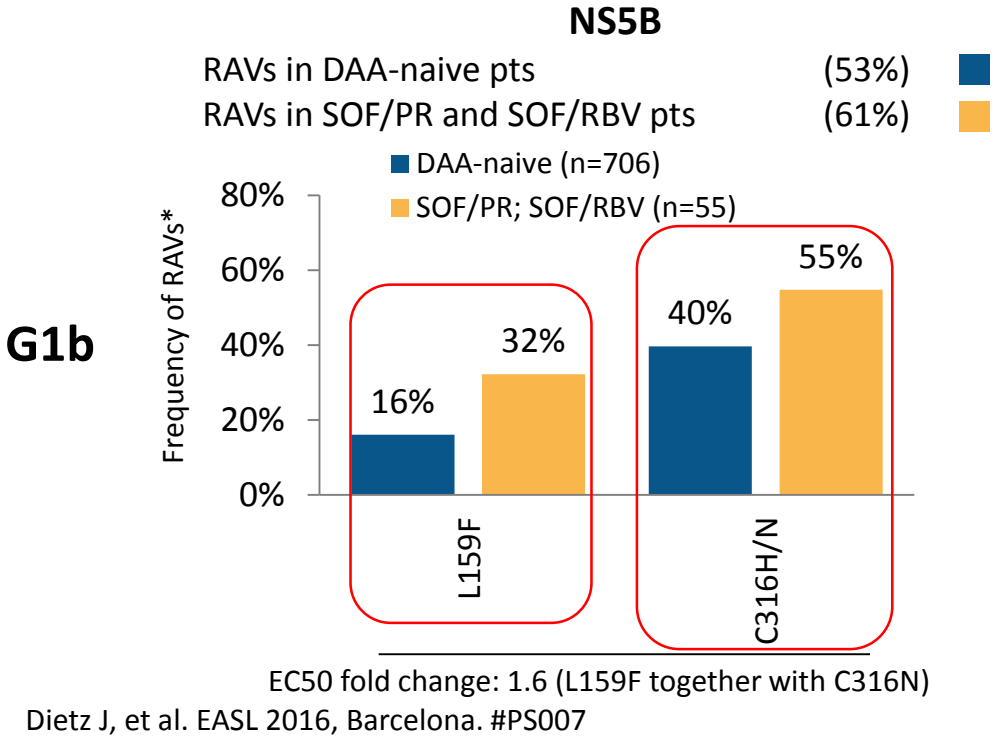
Date on file, Gilead
Hongmei Mo, personal communication

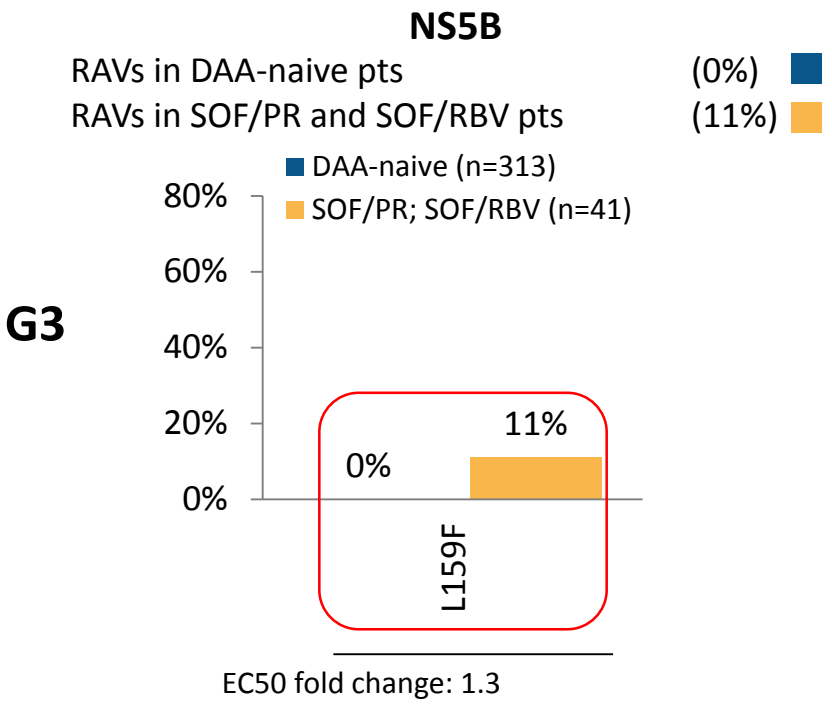
# Frequency and characteristics of RAVs in treatment-naive and DAA-experienced patients - European RAVs database:

- Serum samples of 3549 European HCV-infected patients
- Population-based sequencing for NS3, NS5A, and NS5B
  - Considered relevant if associated with treatment failure or shown to confer >2-fold changed drug susceptibility in comparison to reference strain

**RAVs in SOF/PR and SOF/RBV failures**

- No RAVs detected in G1a or G2



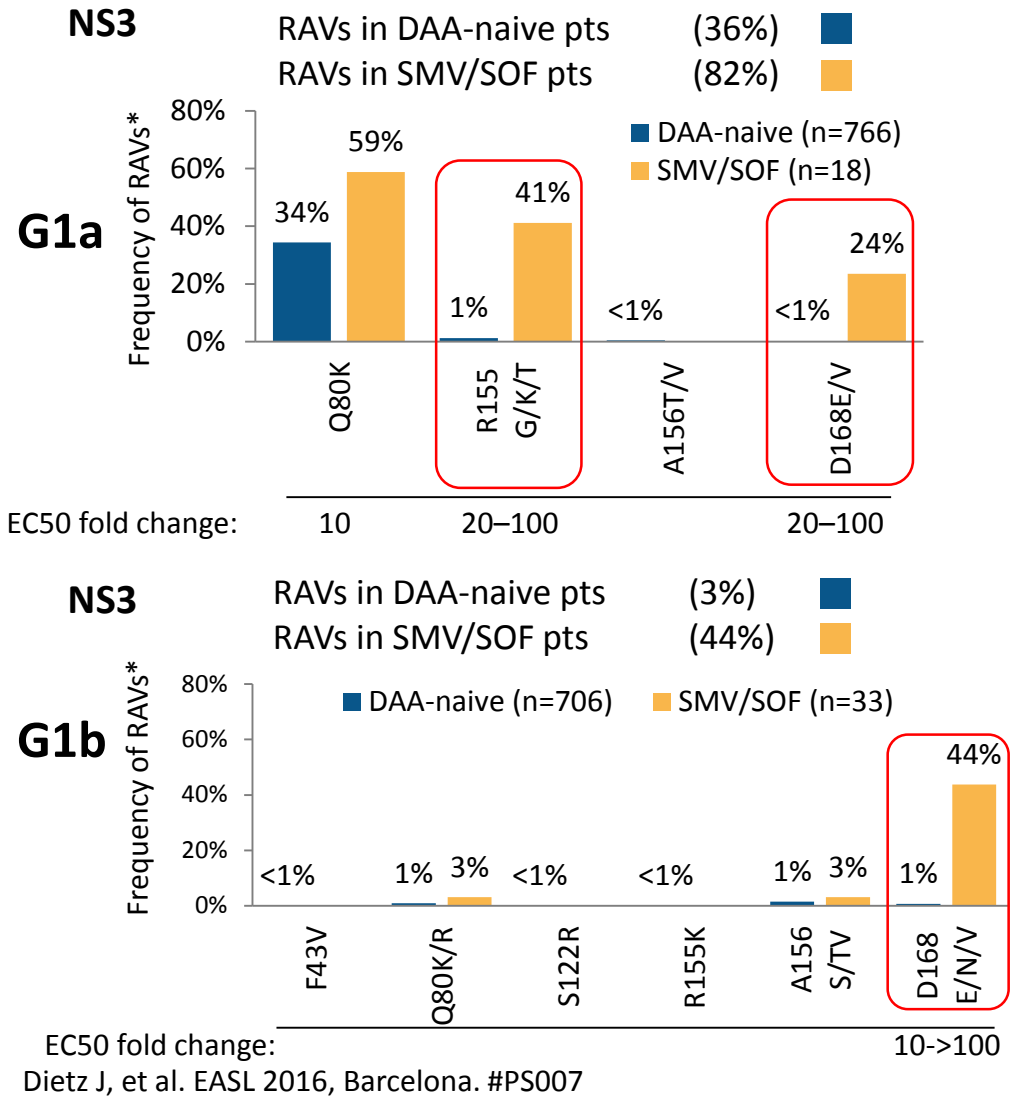



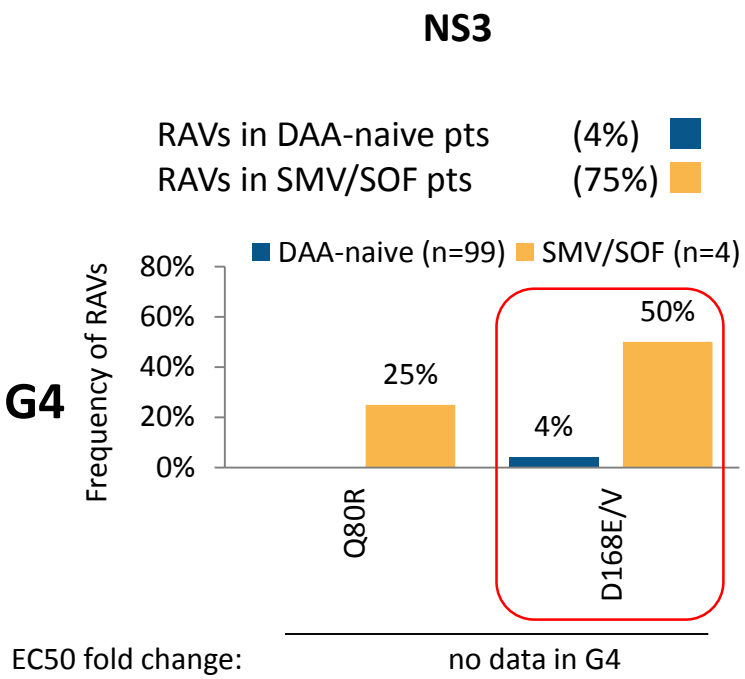

Dietz J, et al. EASL 2016, Barcelona. #PS007

*Double variants were calculated as single RAVs. Thus, sum of single RAVs frequencies is not identical with rate of patients with RAVs.

# Frequency and characteristics of RAVs in treatment-naive and DAA-experienced patients - European RAVs database:

## RAVs in SMV/SOF failures

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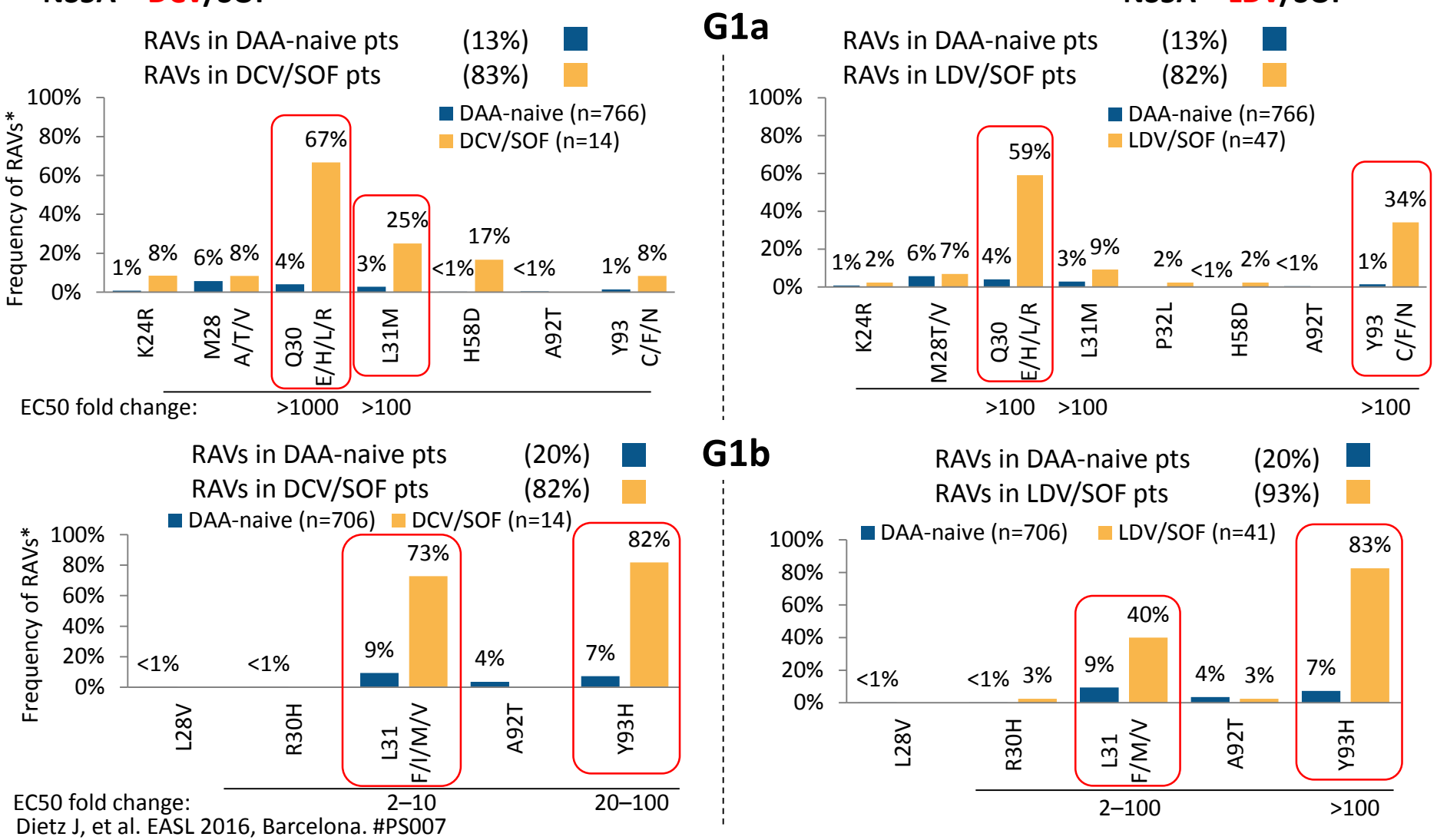
# Frequency and characteristics of RAVs in treatment-naive and DAA-experienced patients - European RAVs database:

## RAVs in DCV/SOF versus LDV/SOF failures (G1)

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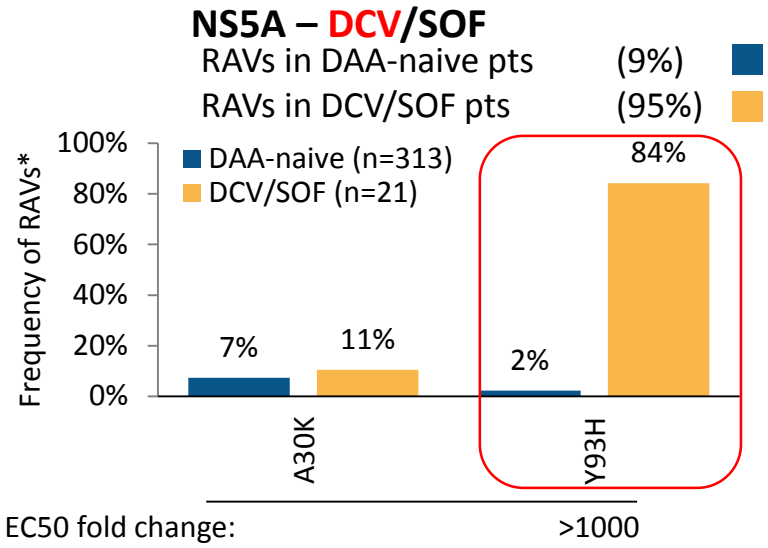
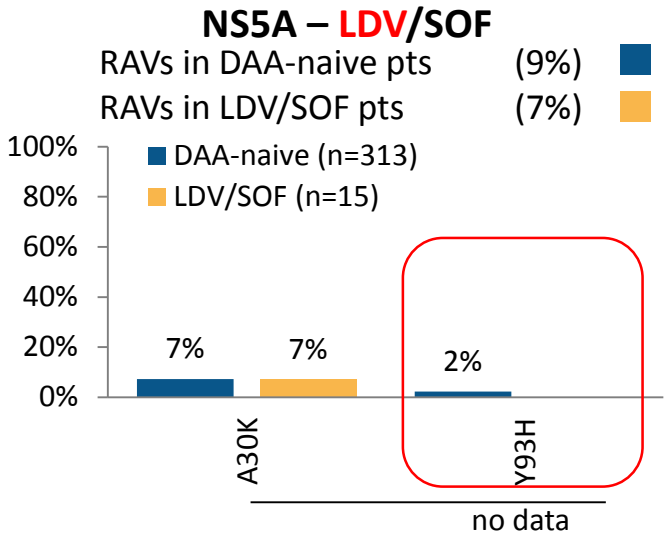
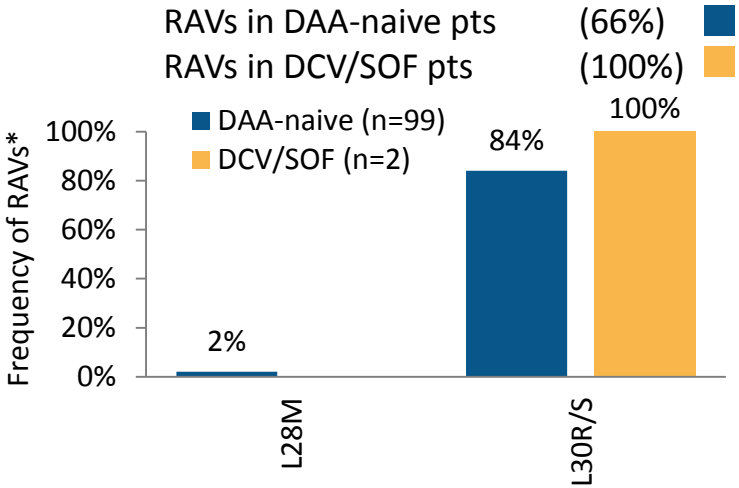
# Frequency and characteristics of RAVs in treatment-naive and DAA-experienced patients - European RAVs database:

## RAVs in DCV/SOF versus LDV/SOF failures (G3 & 4)

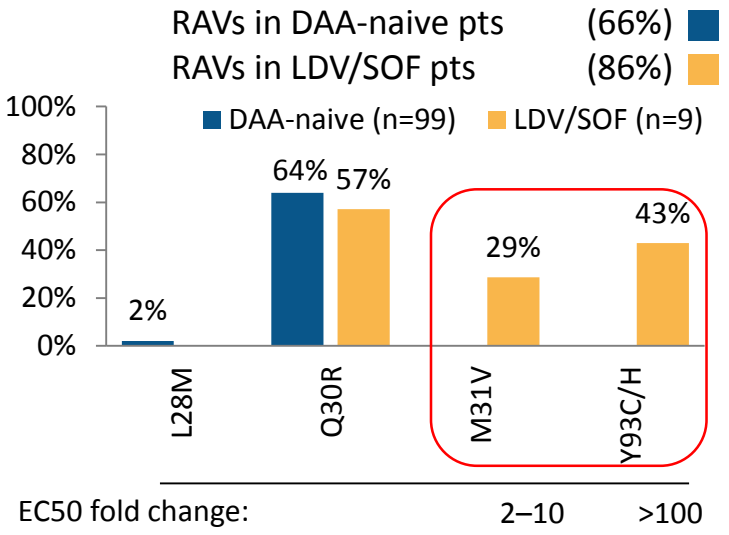



G3




G4




Dietz J, et al. EASL 2016, Barcelona. #PS007

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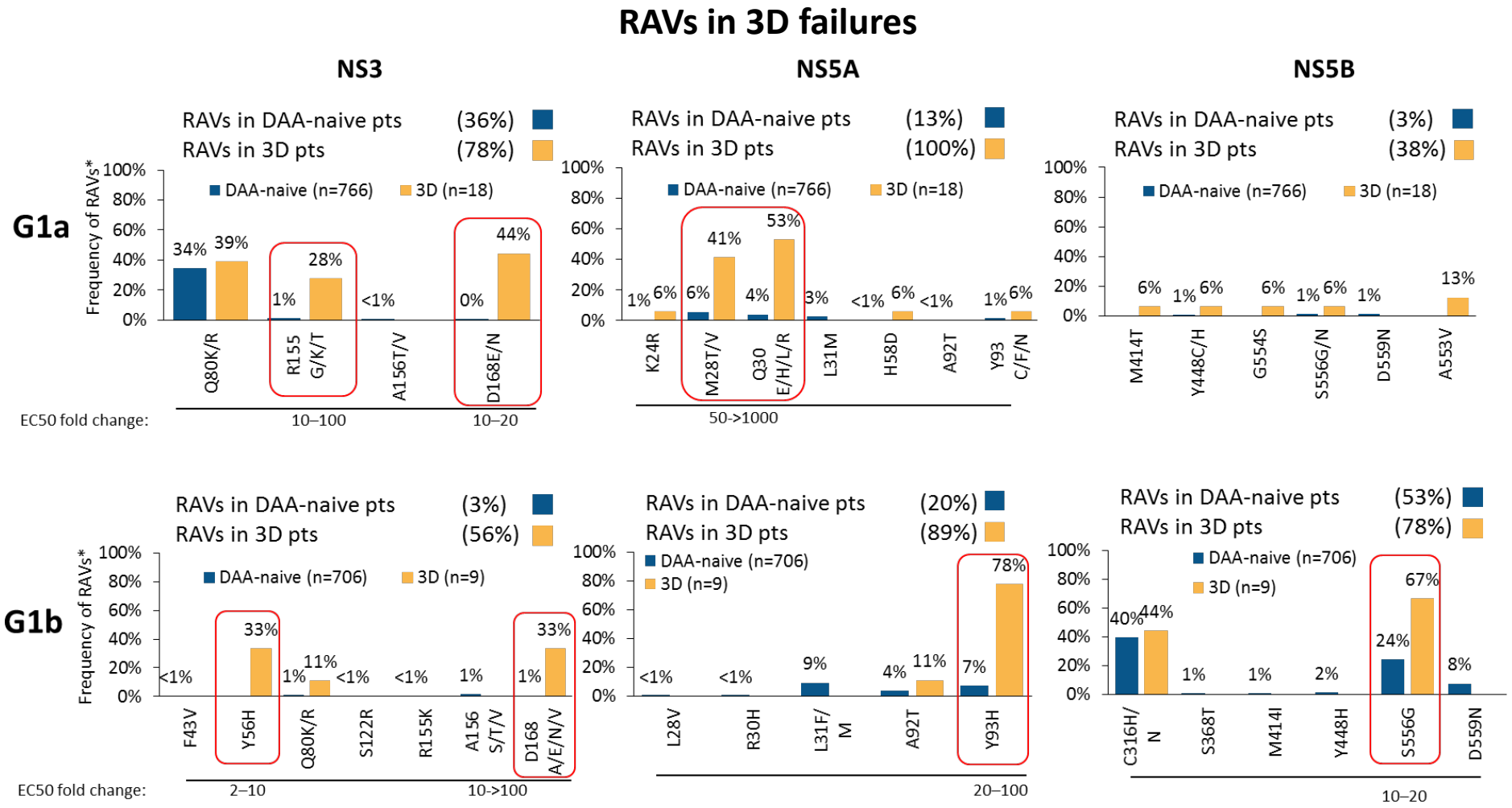


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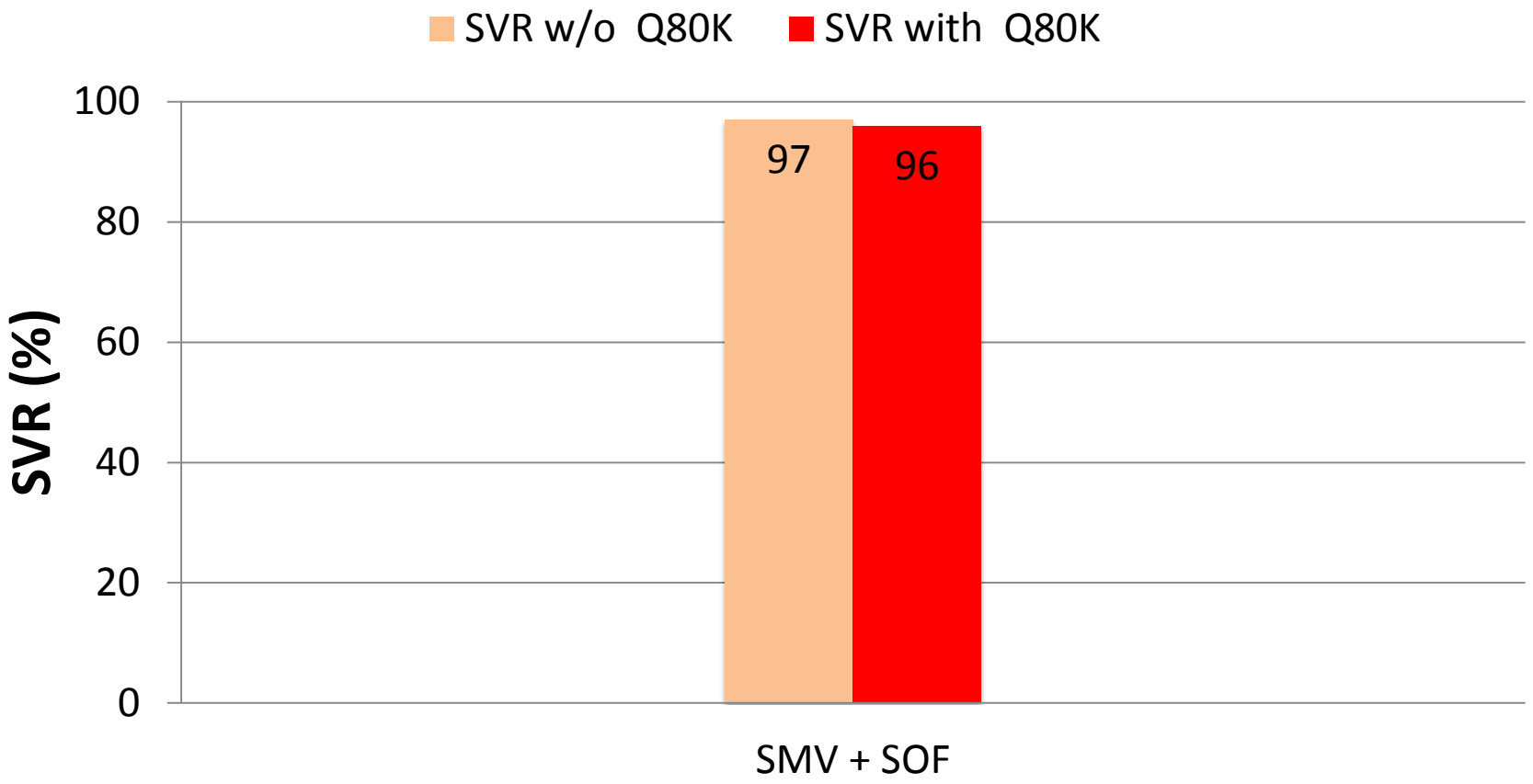
  (e.g. combination partner, treatment duration, stage of disease)

# Simeprevir + Sofosbuvir in GT1a-infected patients (TN+TE): Impact of the NS3 RAV Q80K




| | | | |
|---|---|---|---|
| **Tx duration** | | 12 weeks | |
| **Cirrhosis** | | No | |
| Q80K frequency | | 40% | |

Kwo et al., OPTIMIST-1, EASL 2015; Lawitz et al. , OPTIMIST-2, Hepatology 2015

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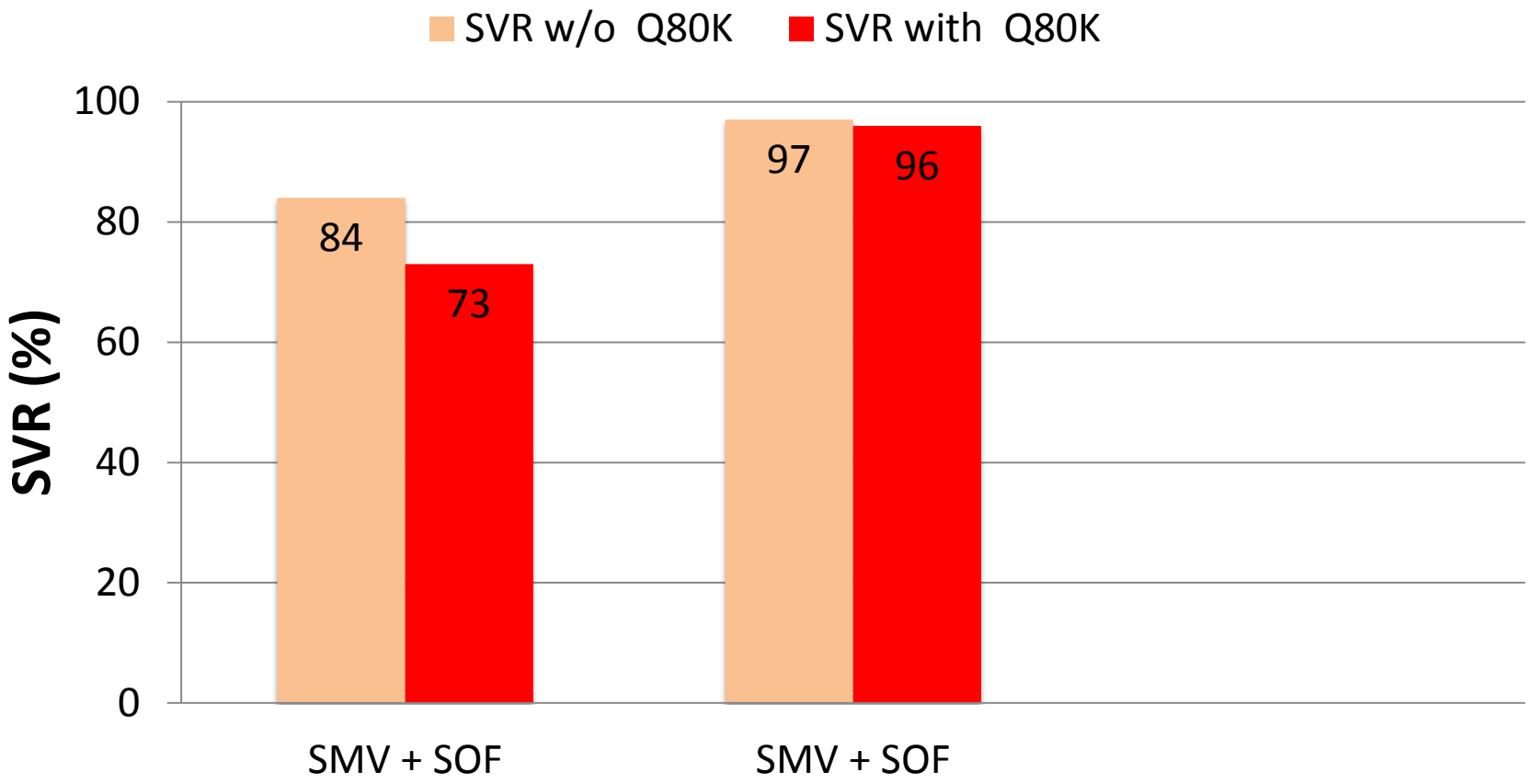


| **Tx duration** | **8 weeks** | 12 weeks | |
|---|---|---|---|
| **Cirrhosis** | No | No | |
| Q80K frequency | 42% | 40% | |

Kwo et al., OPTIMIST-1, EASL 2015; Lawitz et al. , OPTIMIST-2, Hepatology 2015

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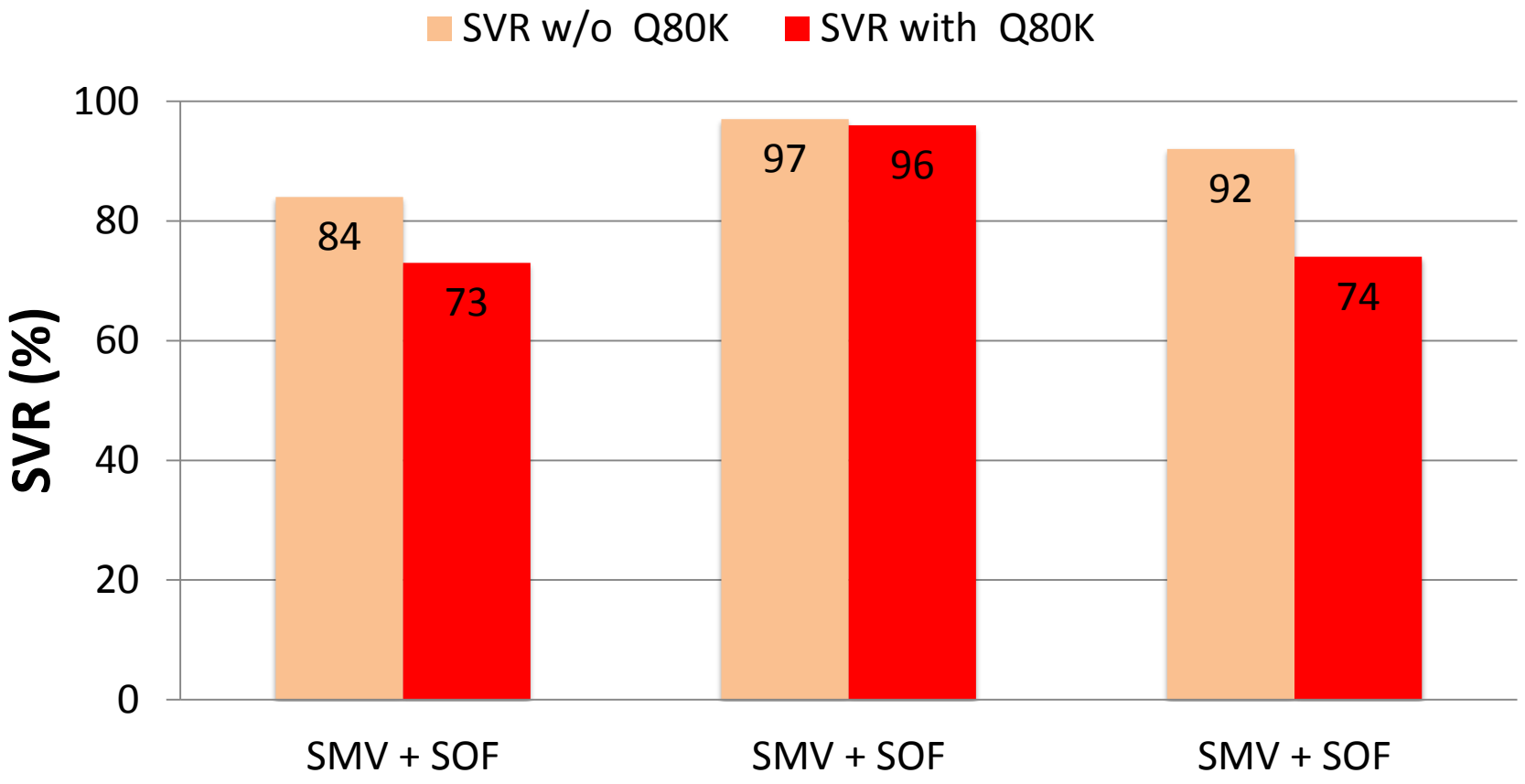
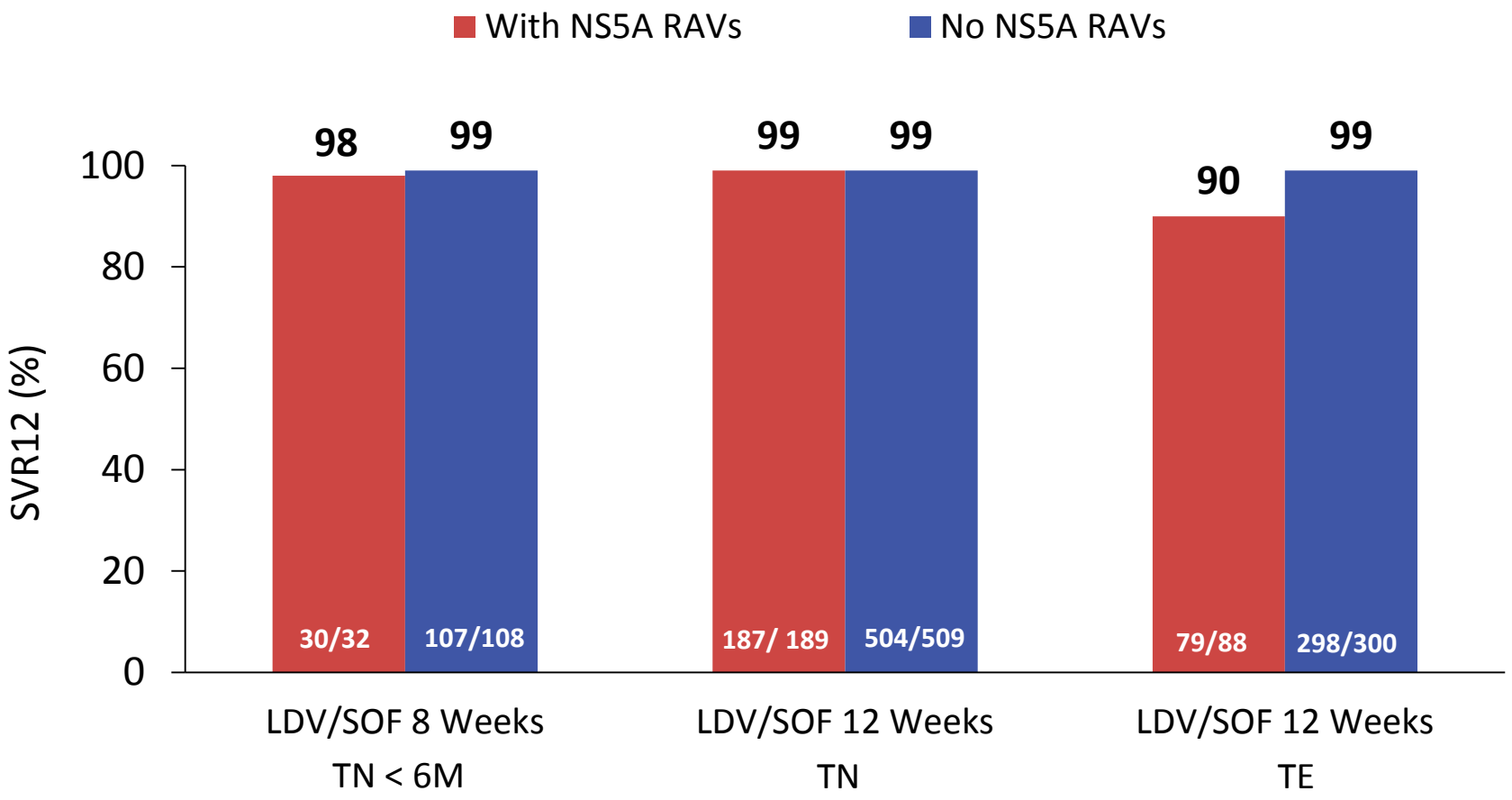



| **Tx duration** | **8 weeks** | 12 weeks | 12 weeks |
|---|---|---|---|
| **Cirrhosis** | No | No | **Yes** |
| Q80K frequency | 42% | 40% | 47% |

Kwo et al., OPTIMIST-1, EASL 2015; Lawitz et al. , OPTIMIST-2, Hepatology 2015

# SVR12 Rates by Treatment Regimen (LDV/SOF)and Duration: Patients without Cirrhosis

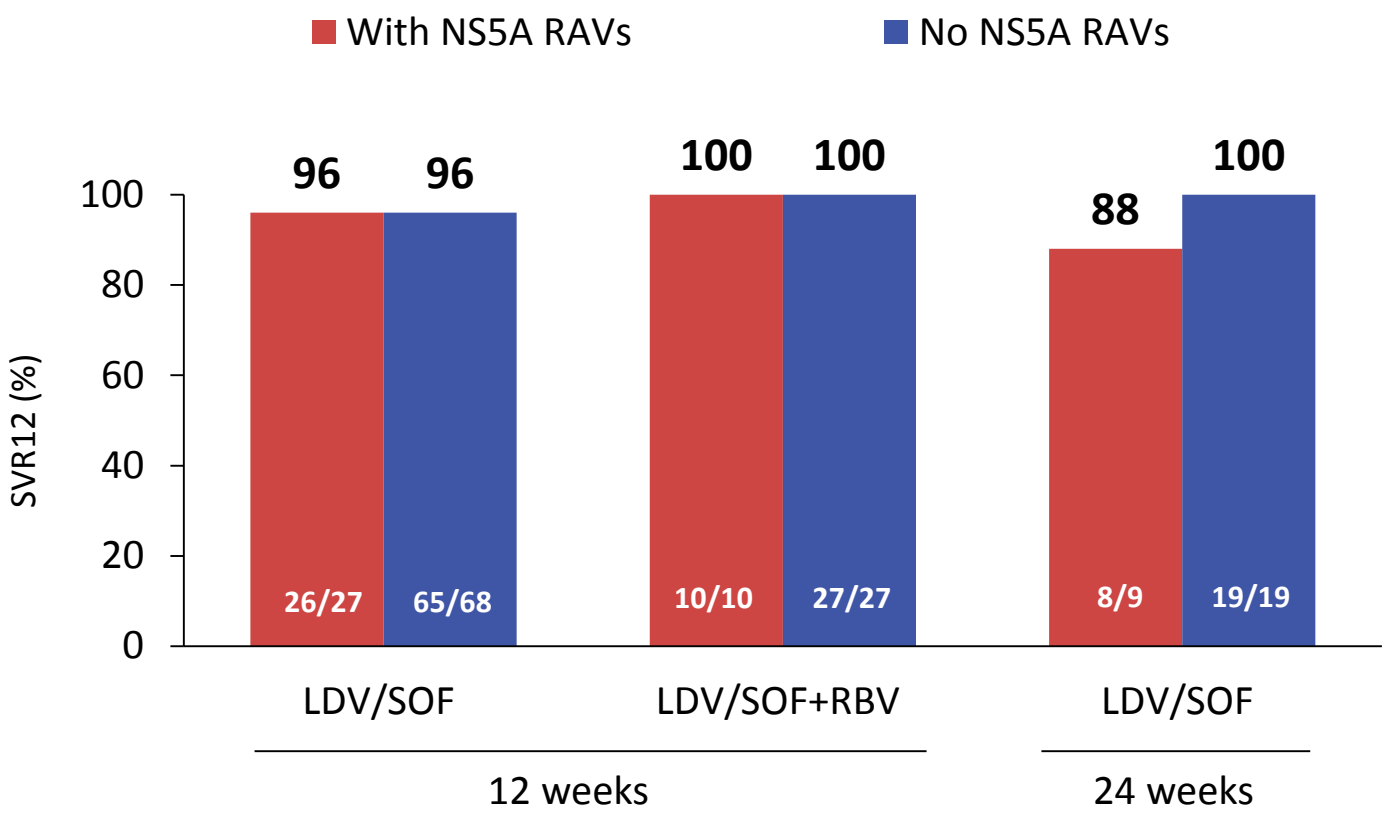



Studies included for analysis:
**LDV/SOF 8 weeks**: GS-US-337-0118 (LONESTAR 1), GS-US-337-0108 (ION-3); **LDV/SOF 12 Wks TN**: GS-US-GS-US-334-1274 (Bleeding Disorder), GS-US-337-0102 (ION-1), GS-US-337-0108 (ION-3), GS-US-337-0113 (Japan 1), GS-US-337-0115 (ION-4), GS-US-337-0122 (Electron 2), GS-US-337-0131(China), GS-US-337-0118 (LONESTAR 1), GS-US-337-1406, GS-US-337-1468 (LEPTON); **LDV/SOF 12 Wks TE**: GS-US-337-0109 (ION-2), GS-US-337-0113 (Japan 1), GS-US-337-0115 (ION-4), GS-US-337-0124 (SOLAR-2), GS-US-334-1274 (Bleeding Disorder), GS-US-337-0118 (LONESTAR 1) , GS-US-337-0131(China), GS-US-337-1406, GS-US-337-1468 (LEPTON)

Sensitivity threshold at 1% (deep sequencing)

Zeuzem et al., AASLD 2015

# SVR12 Rates by Treatment Regimen (LDV/SOF) and Duration: TN Patients with Cirrhosis

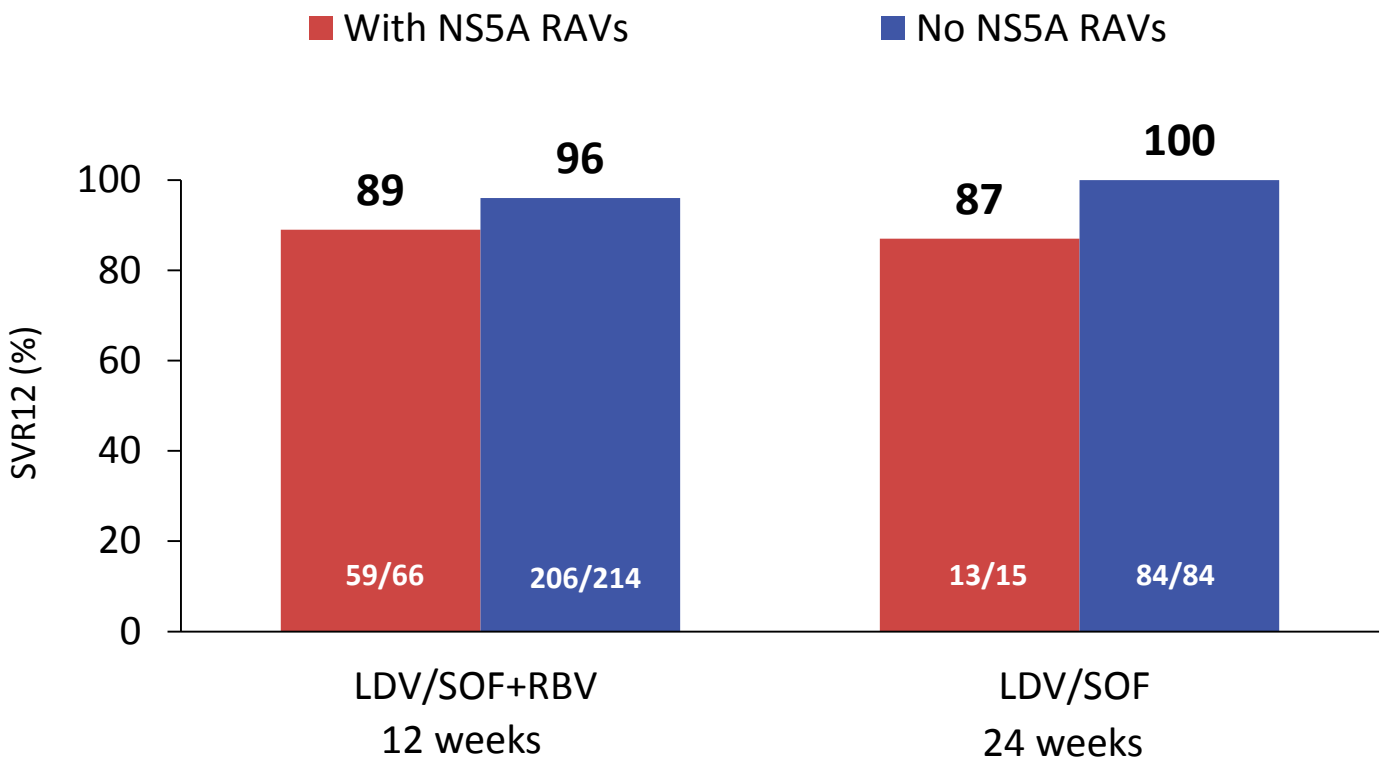



Studies included for analysis:
**LDV/SOF 12 Wks**: GS-US-334-1274 (Bleeding Disorder), GS-US-337-0102 (ION-1), GS-US-337-0113 (Japan 1), GS-US-337-0115 (ION-4), GS-US-337-0122 (ELECTRON-2), GS-US-337-0131(China), GS-US-337-1406; **LDV/SOF+RBV 12 Wks**: GS-US-337-0102 (ION-1), GS-US-337-0113 (Japan 1), GS-US-337-0122 (ELECTRON-2); **LDV/SOF 24 Wks**: GS-US-337-0102 (ION-1), GS-US-334-1274 (Bleeding Disorder)

Sensitivity threshold at 1% (deep sequencing)

Zeuzem et al., AASLD 2015

# SVR12 Rates by Treatment Regimen and Duration: TE Patients with Cirrhosis




Studies included for analysis:
**LDV/SOF+RBV 12 Wks**: GS-US-337-0109 (ION-2), GS-US-337-0113 (Japan 1), GS-US-337-0118 (LONESTAR-1), GS-US-337-0122 (ELECTRON-2), GS-US-337-0123 (SOLAR-1), GS-US-337-0124 (SOLAR-2), GS-US-337-1118 (Retreatment), P7977-0523 (ELECTRON); **LDV/SOF 24 Wks**: GS-US-337-0109 (ION-2), GS-US-337-0121 (SIRIUS), GS-US-334-1274 (Bleeding Disorder)

Sensitivity threshold at 1% (deep sequencing)

Zeuzem et al., AASLD 2015

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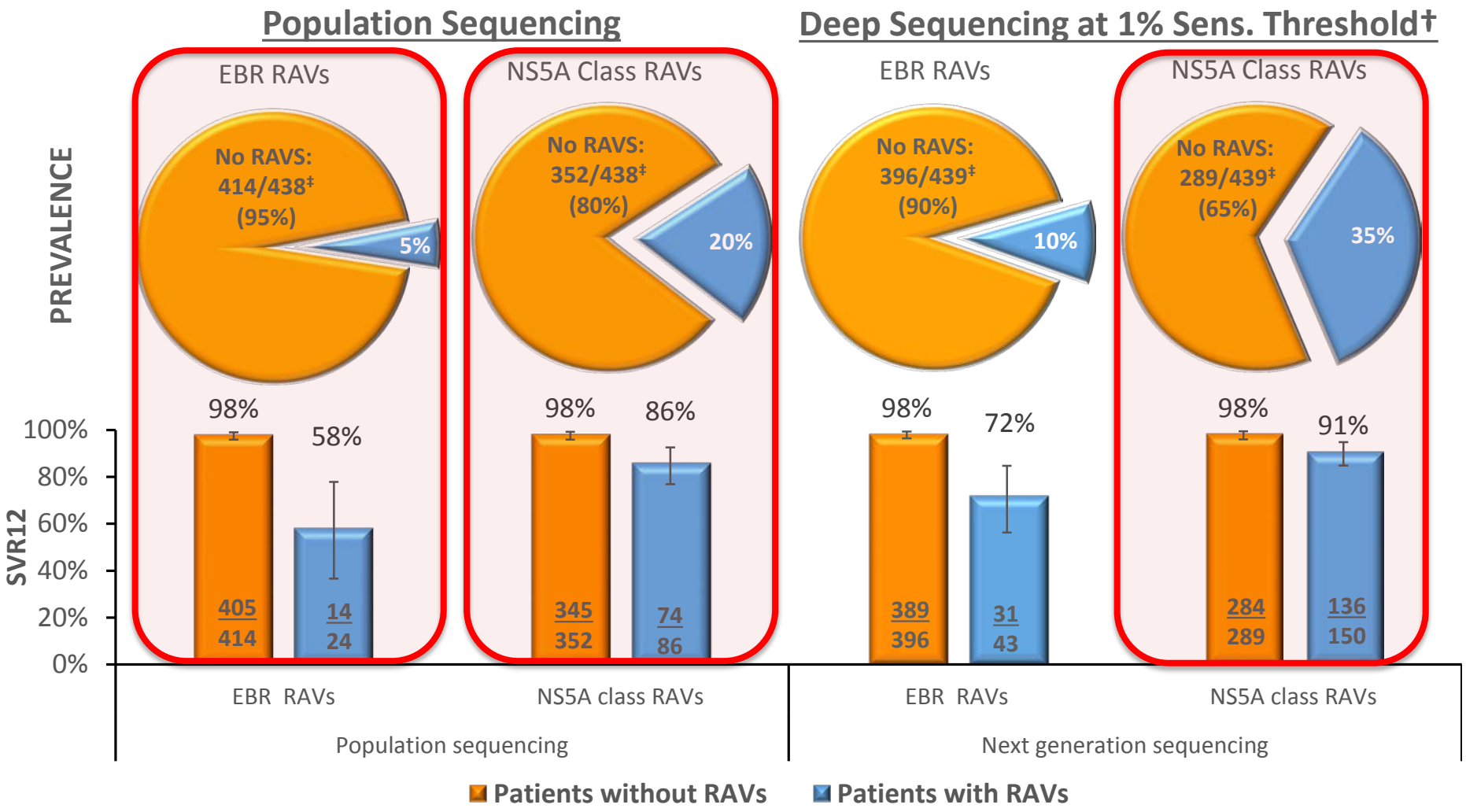
  (e.g. combination partner, treatment duration, stage of disease)

# Grazoprevir + Elbasvir for 12 wks in TN/relapse GT-1a patients: Impact of NS5A RAVs on SVR rates




†NGS with 1% ST supplemented by Population Sequencing when NGS not available. ‡ One GT1a was missing baseline population sequencing data but had baseline NGS data
EBR RAV List = For GT1a: M/L28T/A/G, Q/R30E/H/R/G/K/L/D, L31M/V/F, H58D, or Y93C/H/N/S
NS5A Class RAV List = Any variant from reference strain at NS5A position 24, 28, 30, 31, 32, 38, 58, 92 or 93

Jacobson et al., AASLD 2015

# Effect of RAVs at Specific Baseline Positions on Likelihood to Achieve $SVR_{12}$

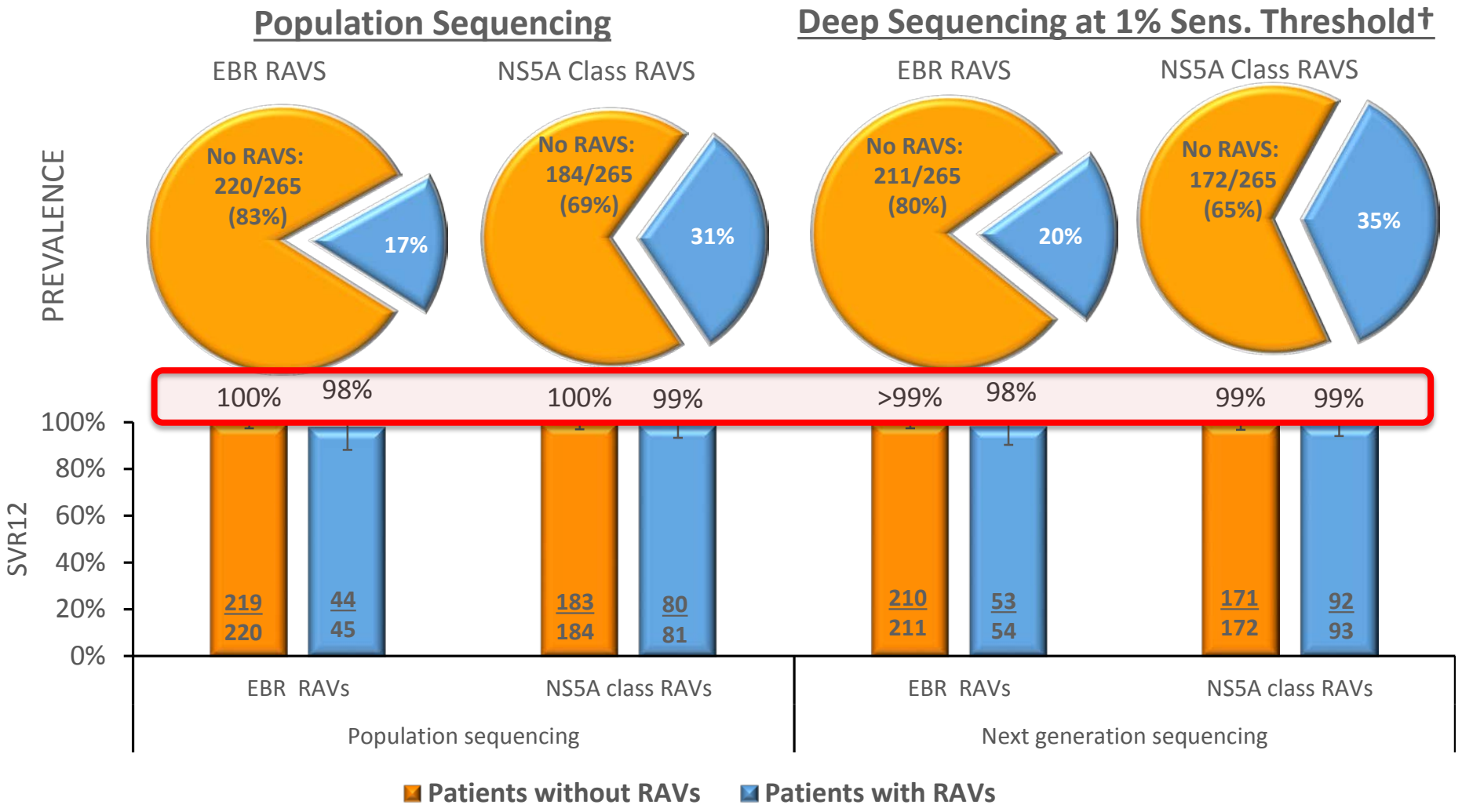
***GT1a-Infected TN/TE Subjects given EBR/GZR 12 weeks (no RBV)***

| RAV Position | SVR12 Subjects with RAVs (1% ST NGS) | SVR12 Subjects With RAVs (Population Sequencing) |
|---|---|---|
| 24 | 15/18 (83.3%) | 4/4 (100.0%) |
| 28 | 61/68 (89.7%) | 29/33 (87.9%) |
| 30 | 14/23 (60.9%) | 4/10 (40.0%) |
| 31 | 15/23 (65.2%) | 5/13 (38.5%) |
| 32 | 1/1 (100.0%) | -- |
| 38 | 9/9 (100.0%) | -- |
| 58 | 75/77 (97.4%) | 48/49 (98.0%) |
| 92 | 6/6 (100.0%) | 3/3 (100.0%) |
| 93 | 9/14 (64.3%) | 5/8 (62.5%) |

NGS using 1% ST supplemented by Population Sequencing when NGS not available

NS5A Class RAV List = Any variant from reference strain at NS5A position 24, 28, 30, 31, 32, 38, 58, 92 and 93

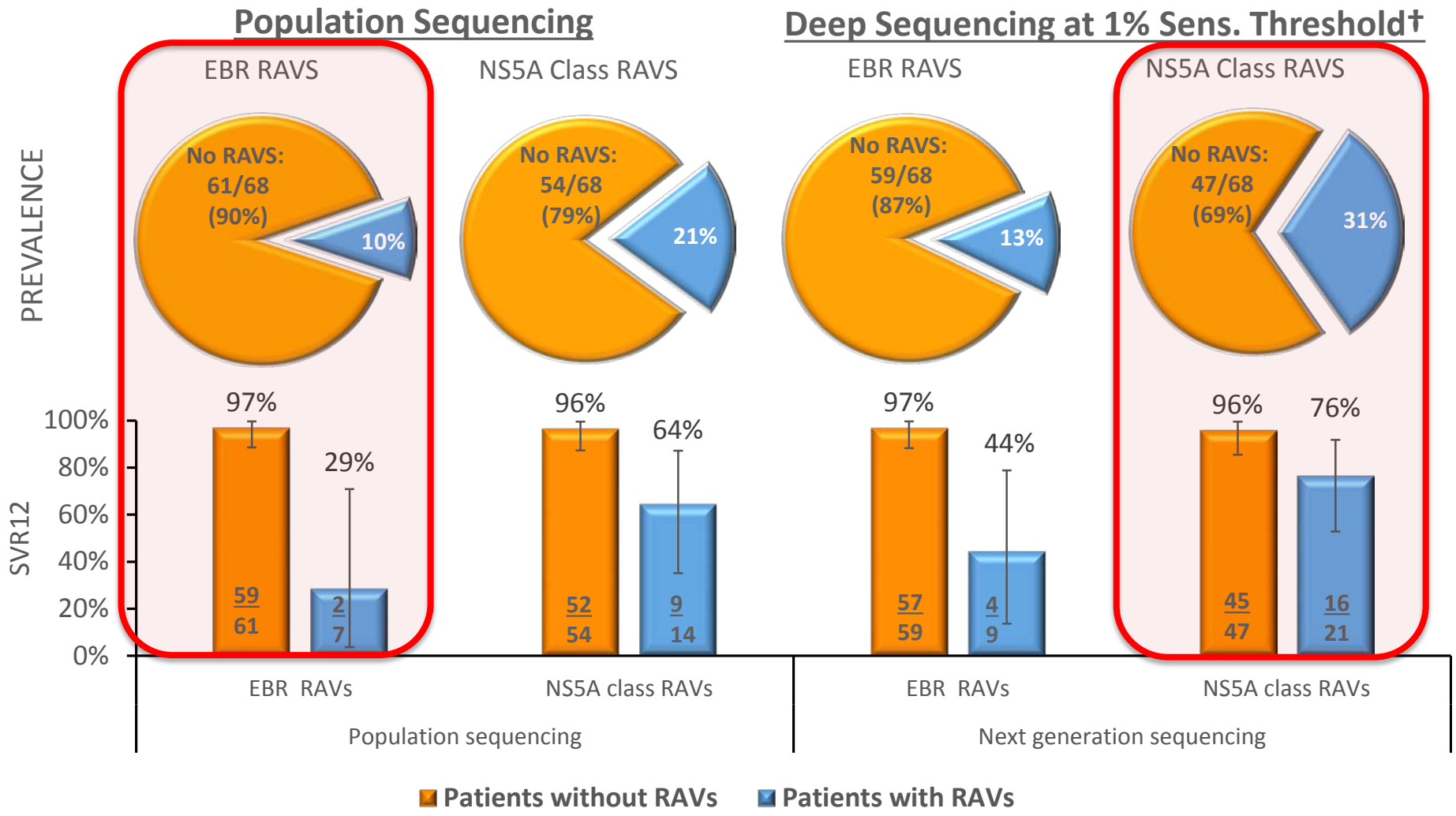
# Grazoprevir + Elbasvir for 12 wks in TN/relapse GT-1b patients: Impact of NS5A RAVs on SVR rates




†NGS 1% ST supplemented by Population Sequencing when NGS not available
EBR RAV List = For GT1b: P32L, P58D or A92K or any variant at NS5A position 28, 30, 31 or 93
NS5A Class RAV List = Any variant from reference strain at NS5A position 24, 28, 30, 31, 32, 38, 58, 92 or 93

Jacobson et al., AASLD 2015

# Grazoprevir + Elbasvir for 12 wks in non-responder GT-1a patients: Impact of NS5A RAVs on SVR rates

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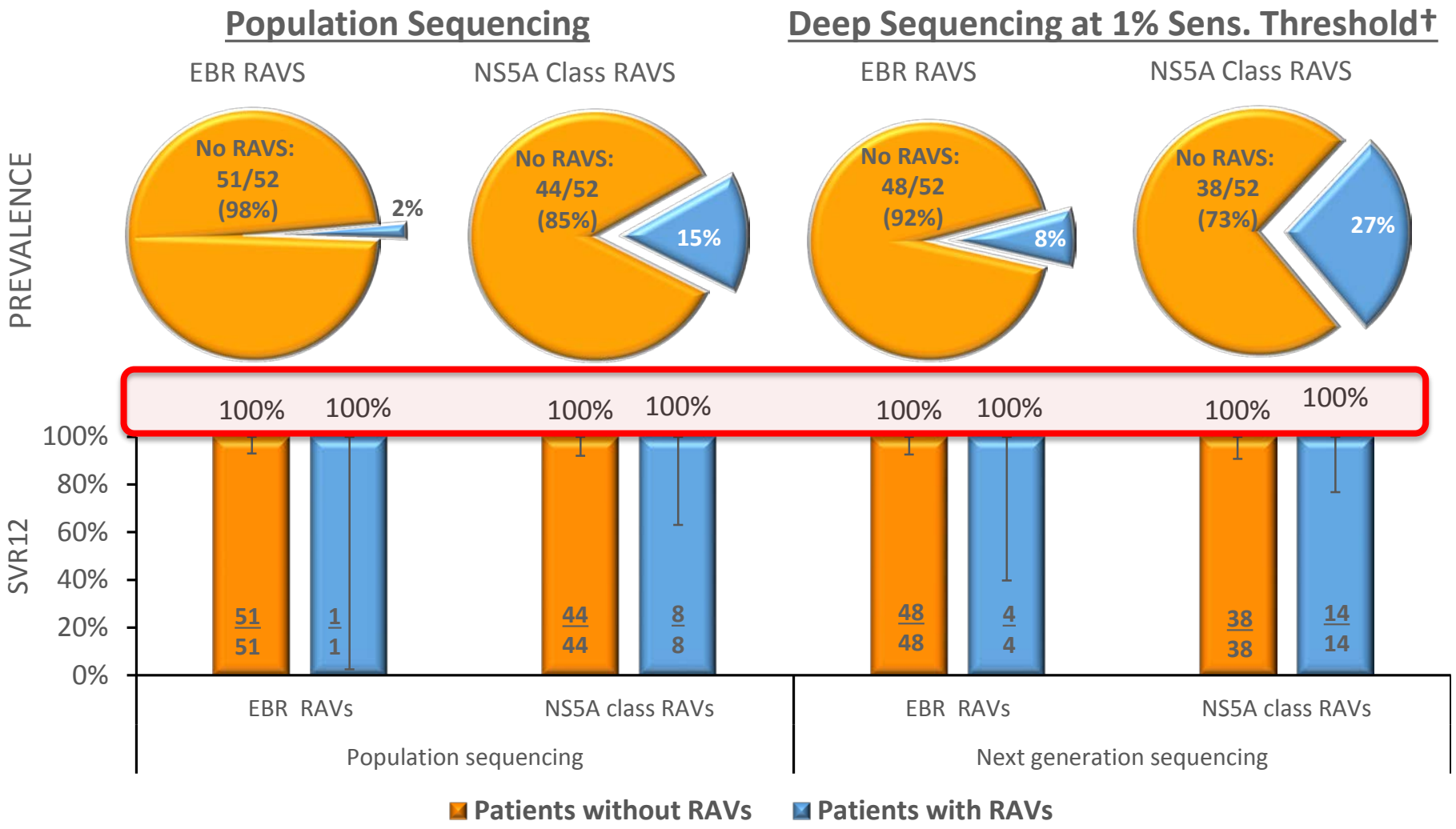
Jacobson et al., AASLD 2015

# Grazoprevir + Elbasvir + RBV for 16/18 wks in NR GT-1a patients: Impact of NS5A RAVs on SVR rates

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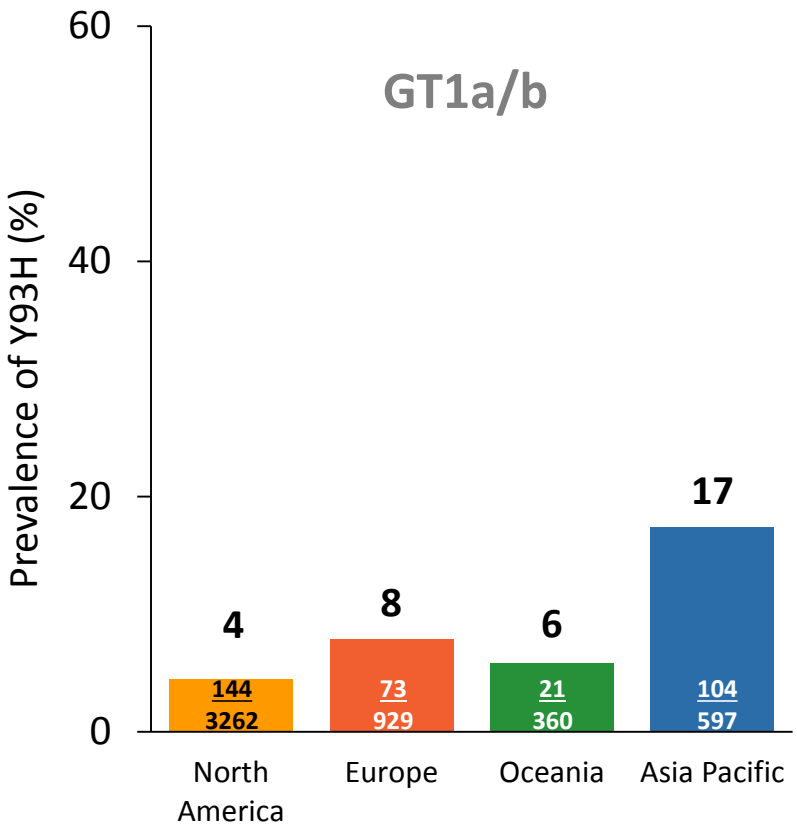
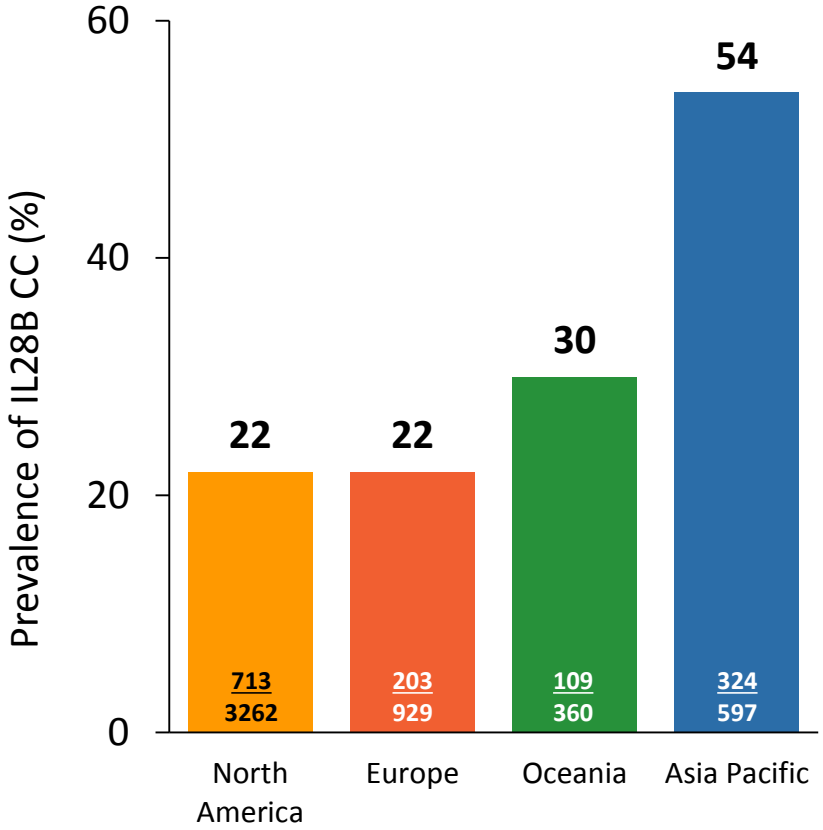
Jacobson et al., AASLD 2015

# How to define a RAV best clinically?

- RAVs (single and combination) per specific drug
- Genotypic backbone *in vitro* and *in vivo*
- Level of reduced susceptibility *in vitro* >100-fold
- sensitivity threshold at 15-25% (population sequencing)
- Impact of RAVs on SVR rates in defined patient population and a defined regimen (DAA combination, treatment duration)
- and there is even more complexity .....

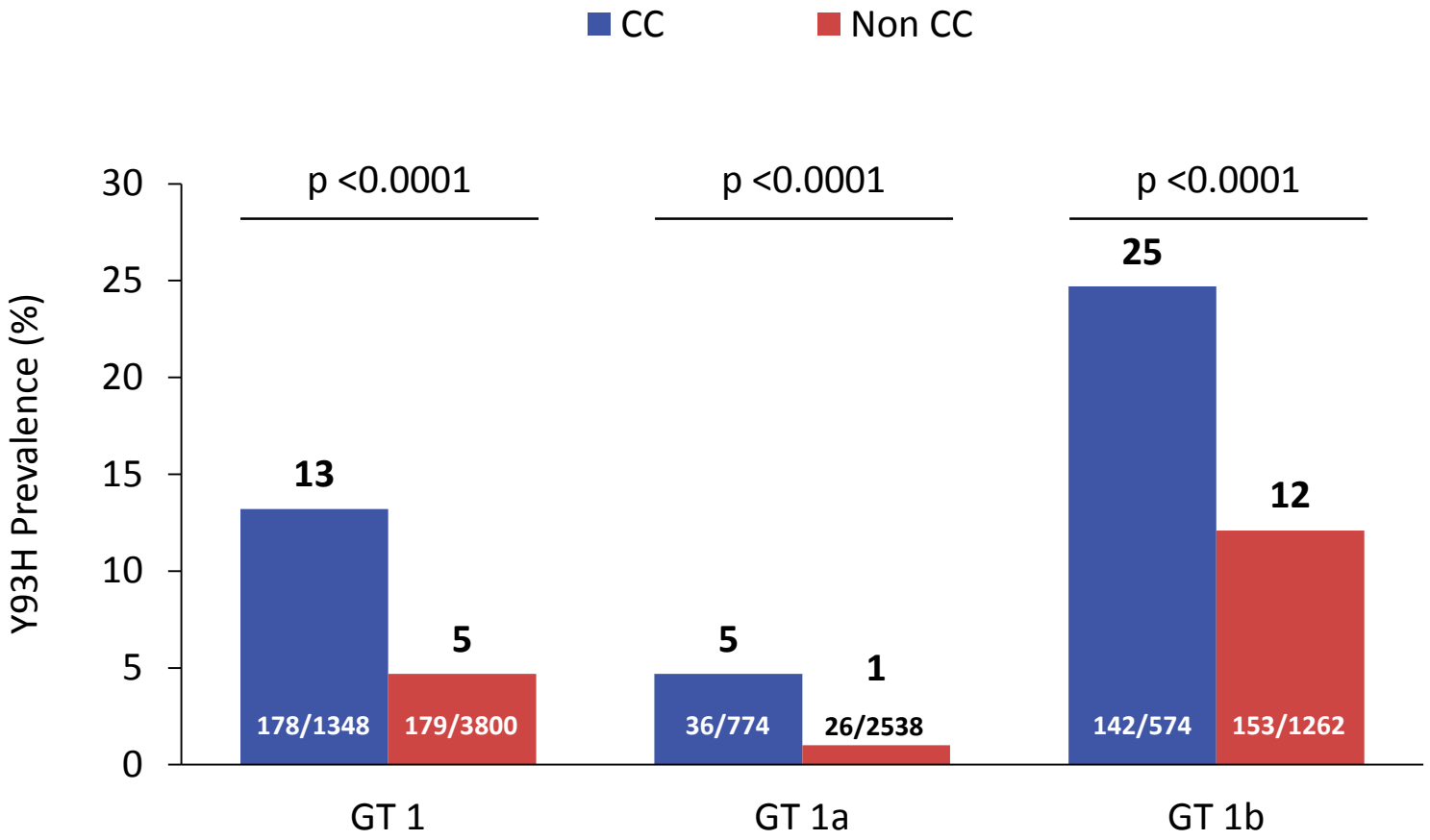
# Y93H and IL28B CC prevalence by region




Zeuzem et al., AASLD 2015

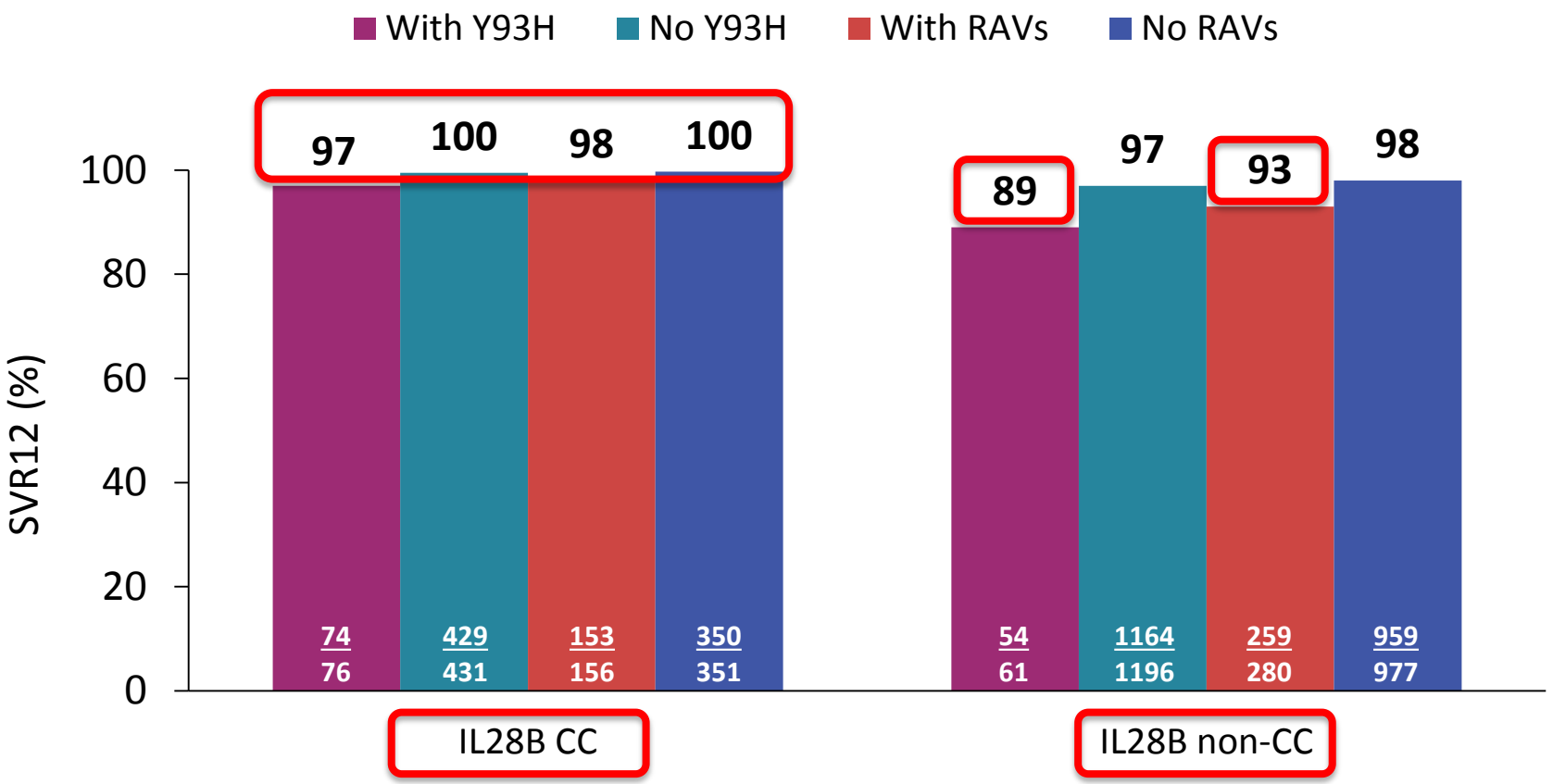
# Association of Y93H and IL28B genotype




- 5,148 GT1 patients in Gilead HCV clinical trials with both NS5A sequencing and IL28B genotype data

Zeuzem et al., AASLD 2015

# SVR12 rates by Y93H or any NS5A RAV and IL28B genotype

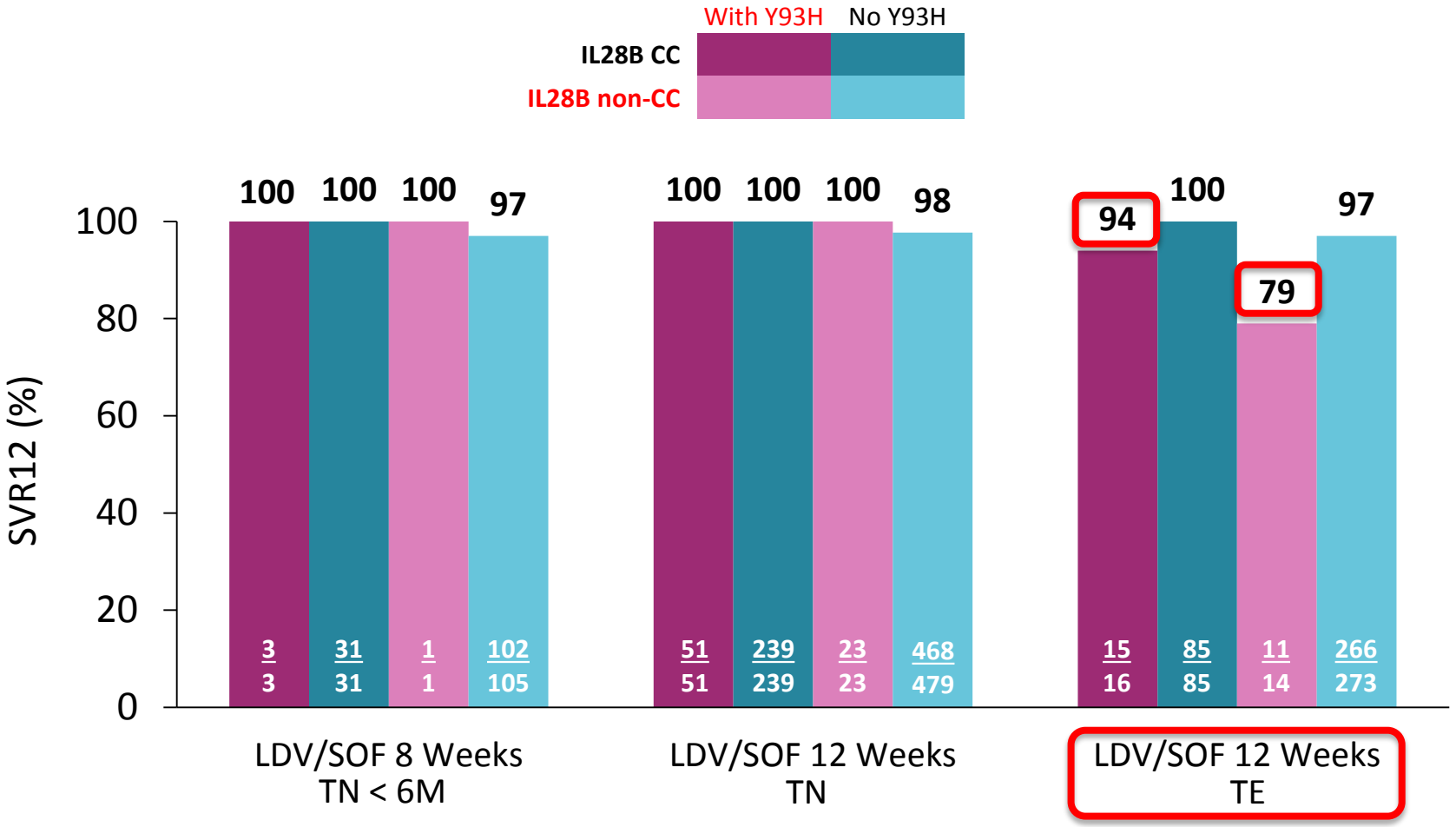



♦ SVR12 data pooled from all guideline recommended LDV/SOF regimens

Studies included for analysis:
GS-US-337-0118 (LONESTAR 1), GS-US-GS-US-334-1274 (Bleeding Disorder), GS-US-337-0102 (ION-1), GS-US-337-0108 (ION-3), GS-US-337-0113 (Japan 1), GS-US-337-0115 (ION-4), GS-US-337-0122 (Electron 2), GS-US-337-0131(China), GS-US-337-1406, GS-US-337-1468 (LEPTON), GS-US-337-0109 (ION-2), GS-US-337-0124 (SOLAR-2), GS-US-334-1274 (Bleeding Disorder), GS-US-337-1406

Zeuzem et al., AASLD 2015

# SVR12 Rates by Treatment Regimen and Y93H/IL28B: Patients without Cirrhosis




Studies included for analysis:
GS-US-337-0118 (LONESTAR 1), GS-US-GS-US-334-1274 (Bleeding Disorder), GS-US-337-0102 (ION-1), GS-US-337-0108 (ION-3), GS-US-337-0113 (Japan 1), GS-US-337-0115 (ION-4), GS-US-337-0122 (Electron 2), GS-US-337-0131(China), GS-US-337-1406, GS-US-337-1468 (LEPTON), GS-US-337-0109 (ION-2), GS-US-337-0124 (SOLAR-2), GS-US-334-1274 (Bleeding Disorder), GS-US-337-1406

# It is useful to detect RAVs ?

- There is no “YES” or “No” answer
- First and most important: Methodology for detection of RAVs must standardized (and automated)
- Pharmaceutical industry must fully publish available RAV data in collaboration with academia
- Usefulness of RAV testing will be patient population and treatment regimen dependent
- RAV testing most likely not required in patient populations with SVR rates > 99%
- RAV testing most likely be clinical useful and cost-effective in population with suboptimal SVR rates (definition < 95% / < 90% ?) & if population large enough
  - Regimens w/o a very high barrier to resistance drug
  - Treatment-experienced patients (in particular when exposed to DAAs)
  - Patients with cirrhosis
  - When the shortest possible treatment duration is economically important
- IL28B CC genotype is significantly associated with a higher prevalence of Y93H. This association is functionally not yet understood
- The relevance of (in particular NS5A) RAVs, baseline HCV RNA levels, and IL28B genotype on treatment outcome requires further intensive clinical research

# Treatment decisions in patients with RAVs

- Switch drug class
  (exception: nucleosidic polymerase inhibitors)
- Treat longer (up to 24 weeks)
- Add ribavirin
- Use triple therapies (NI + PI + NS5A-Inhibitor)
- Second generation PIs and NS5A-Inhibitors
  (e.g. Glecaprevir, Pibrentasvir)
- Peginterferon (??)